PCT WORLD INTELLECTUAL PROP
International B

INTERNATIONAL APPLICATION PUBLISHED UNDEI

WO 9607917A1

(51) International Patent Classification 6 :
G01N 35/00, C12P 19/34, C12M 1/00

A1

(11) International Publication Number: WO 96/07917

(43) International Publication Date: 14 March 1996 (14.03.96)

(21) International Application Number: PCT/US95/11333

(22) International Filing Date: 6 September 1995 (06.09.95)

(30) Priority Data:
08/304,657 9 September 1994 (09.09.94) US

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(81) Designated States: AU, BR, CA, CN, FI, JP, NZ, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: AUTOMATED MOLECULAR BIOLOGICAL DIAGNOSTIC SYSTEM

(57) Abstract

Self-addressable, self-assembling microelectronic system for performing molecular diagnosis, analysis, multi-step and multiplex reactions in microscopic formats. Actively controlled reactions include nucleic acid hybridization, immunoassays, clinical diagnosis and multi-step combinatorial biopolymer synthesis. Controller interfaces with user via input/output devices preferably including a graphical display. The controller may interface with a power supply and interface, the interface providing selective connection to individual microlocations, polarity reversal, and selective potential or current levels to individual electrodes. A combined system for performing DNA sample preparation, hybridization, detection and data analysis integrates multiple steps. Charged materials are transported preferably by free field electrophoresis. DNA complexity reduction is preferably achieved by binding DNA to a support, cleaving unbound materials such as by restriction enzymes, and transporting the cleaved fragments. Active, programmable matrix devices include a square matrix pattern with fanned out electrical connections and optional electrical connections beneath specific microlocations resulting in a highly automated DNA diagnostic system.

DESCRIPTION

Automated Molecular Biological Diagnostic System

Field of the Invention

This invention relates to devices and systems for performing multi-step molecular biological type diagnostic analyses in multiplex formats. More particularly, the molecular biological type analyses include various nucleic acid hybridizations reactions and associated biopolymer synthesis. Additionally, antibody/antigen reactions and other clinical diagnostics can be performed.

Related Application Information

This application is a continuation-in-part of application Serial No. 08/271,882, filed July 7, 1994, which is a continuation-in-part of Serial No. 07/146,504, filed November 1, 1993, both entitled "SELF-ADDRESSABLE SELF-ASSEMBLING MICROELECTRIC SYSTEMS AND DEVICES FOR MOLECULAR BIOLOGICAL ANALYSIS AND DIAGNOSTICS."

Background of the Invention

Molecular biology comprises a wide variety of techniques for the analysis of nucleic acid and protein. Many of these techniques and procedures form the basis of clinical diagnostic assays and tests. These techniques include nucleic acid hybridization analysis, restriction enzyme analysis, genetic sequence analysis, and the separation and purification of nucleic acids and proteins (See, e.g., J. Sambrook, E. F. Fritsch, and T. Maniatis, Molecular Cloning: A Laboratory Manual, 2 Ed., Cold spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989).

Most of these techniques involve carrying out numerous operations (e.g., pipetting, centrifugations, electrophoresis)on a large number of samples. They are often complex and time consuming, and generally require a high degree of accuracy. Many a technique is limited in its

actual hybridization reaction is performed. Finally, detection and data analysis convert the hybridization event into an analytical result.

The steps of sample preparation and processing have typically been performed separate and apart from the other main steps of hybridization and detection and analysis. Indeed, the various substeps comprising sample preparation and DNA processing have often been performed as a discrete operation separate and apart from the other substeps. Considering these substeps in more detail, samples have been obtained through any number of means, such as obtaining of full blood, tissue, or other biological fluid samples. In the case of blood, the sample is processed to remove red blood cells and retain the desired nucleated (white) cells. This process is usually carried out by density gradient centrifugation. Cell disruption or lysis is then carried out, preferably by the technique of sonication, freeze/thawing, or by addition of lysing reagents. Crude DNA is then separated from the cellular debris by a centrifugation step. Prior to hybridization, double-stranded DNA is denatured into single-stranded form. Denaturation of the double-stranded DNA has generally been performed by the techniques involving heating (>Tm), changing salt concentration, addition of base (NaOH), or denaturing reagents (urea, formamide, etc.). Workers have suggested denaturing DNA into its single-stranded form in an electrochemical cell. The theory is stated to be that there is electron transfer to the DNA at the interface of an electrode, which effectively weakens the double-stranded structure and results in separation of the strands. See, generally, Stanley, "DNA Denaturation by an Electric Potential", U.K. patent application 2,247,889 published March 18, 1992.

Nucleic acid hybridization analysis generally involves the detection of a very small number of specific target nucleic acids (DNA or RNA) with an excess of probe DNA, among a relatively large amount of complex non-target

July 8, 1987) and for the detection of overlapping clones and the construction of genomic maps (G. A. Evans, in US Patent Number 5,219,726, June 15, 1993).

New techniques are being developed for carrying out multiple sample nucleic acid hybridization analysis on micro-formatted multiplex or matrix devices (e.g., DNA chips) (see M. Barinaga, 253 Science, pp. 1489, 1991; W. Bains, 10 Bio/Technology, pp. 757-758, 1992). These methods usually attach specific DNA sequences to very small specific areas of a solid support, such as micro-wells of a DNA chip. These hybridization formats are micro-scale versions of the conventional "dot blot" and "sandwich" hybridization systems.

The micro-formatted hybridization can be used to carry out "sequencing by hybridization" (SBH) (see M. Barinaga, 253 Science, pp. 1489, 1991; W. Bains, 10 Bio/Technology, pp. 757-758, 1992). SBH makes use of all possible n-nucleotide oligomers (n-mers) to identify n-mers in an unknown DNA sample, which are subsequently aligned by algorithm analysis to produce the DNA sequence (R. Drmanac and R. Crkvenjakov, Yugoslav Patent Application #570/87, 1987; R. Drmanac et al., 4 Genomics, 114, 1989; Strezoska et al., 88 Proc. Natl. Acad. Sci. USA 10089, 1992; and R. Dramanac and R. B. Crkvenjakov, U.S. Patent #5,202,231, April 13, 1993).

There are two formats for carrying out SBH. The first format involves creating an array of all possible n-mers on a support, which is then hybridized with the target sequence. The second format involves attaching the target sequence to a support, which is sequentially probed with all possible n-mers. Both formats have the fundamental problems of direct probe hybridizations and additional difficulties related to multiplex hybridizations.

Southern, United Kingdom Patent Application GB 8810400, 1988; E. M. Southern et al., 13 Genomics 1008, 1992, proposed using the first format to analyze or sequence DNA. Southern identified a known single point

Beattie et al., in The 1992 San Diego Conference: Genetic Recognition, November, 1992, used a microrobotic system to deposit micro-droplets containing specific DNA sequences into individual microfabricated sample wells on a glass substrate.

Generally, the prior art processes have been extremely labor and time intensive. For example, the PCR amplification process is time consuming and adds cost to the diagnostic assay. Multiple steps requiring human intervention either during the process or between processes is suboptimal in that there is a possibility of contamination and operator error. Further, the use of multiple machines or complicated robotic systems for performing the individual processes is often prohibitive except for the largest laboratories, both in terms of the expense and physical space requirements.

As is apparent from the preceding discussion, numerous attempts have been made to provide effective techniques to conduct multi-step, multiplex molecular biological reactions. However, for the reasons stated above, these techniques are "piece-meal" and limited. These various approaches are not easily combined to form a system which can carry out a complete DNA diagnostic assay. Despite the long-recognized need for such a system, no satisfactory solution has been proposed previously.

Summary of the Invention

The present invention relates to the design, fabrication, and uses of a self-addressable self-assembling microelectronic devices and systems which can actively carry out controlled multi-step processing and multiplex reactions in a microscopic formats. These reactions include, but are not limited to, most molecular biological procedures, such as nucleic acid hybridization, antibody/antigen reaction, and related clinical diagnostics. In addition, the claimed devices and systems are able to carry out multi-step combinational biopolymer synthesis,

and analysis. In this fullest form, the sample is first prepared, such as by an electronic cell sorter component. Generally, electronic refers more specifically to the ability of the component device to electrophoretically transport charged entities to or from itself. Further DNA processing and complexity reduction may optionally be performed by a crude DNA selector component, and a restriction fragment selector component. The final processed target DNA is transported to the analytical component where electronic hybridization analysis is carried out in a microscopic multiplex format. This analytical component device is also referred to as the APEX or analytical chip. Associated detection and image analysis components provide the results.

Within the system materials may optionally be transported between components (devices) by free field electrophoresis, channelling, fluidics or other techniques. Optionally, electronic reagent dispenser components can provide electrophoretic transport of reagents to the various processing components of the system. Optionally, an electronic waste disposal system may be formed by providing an electrode and charged matrix material that attracts and holds charged waste products. Optionally, an electronic DNA fragment storage system can serve to temporarialy hold other DNA fragments for later hybridization analysis.

In one aspect of this invention, genomic DNA complexity reduction is performed by processes that isolate those specific DNA fragments containing the desired target sequence from the bulk of the DNA material that lacks the desired target sequence. Crude DNA can be transported and captured on a support material. The bound DNA can then be severed using appropriate restriction enzymes. After severing, the DNA fragments can be transported to a component device that selectively hybridizes specific DNA fragments. Those fragments that contain the actual target sequences to be analyzed can be selectively released, via further restriction enzyme cleavage, and transported to

and magnifies the image from the hybridization events occurring on the various micro-locations of the device. The emissions are optically filtered and detected by a charge coupled device (CCD) array or microchannel plate detector. The image is then stored and analyzed. Preferably, the results are displayed to the user on the monitor.

In another aspect of this invention, the hybridization system is formed having a plurality of microlocations formed atop a substrate containing control electronics. Specifically, switching circuits are provided to address individually the microlocations. The electrical connections are made via the backside relative to where sample contact is to be made. Additionally, an optical pathway, such as a waveguide, is disposed beneath the microlocation to permit backside access to the microlocation. Optical excitation, if necessary, may be directed to the microlocation via the waveguide. Detection of emitted radiation may be detected via the backside waveguide. In yet another aspect of this invention, a sample containment system is disposed over the system, particularly the hybridization matrix region. In the preferred embodiment, the matrix hybridization region (including sample containment component) is adapted for removal from the remainder of the device providing the electronic control and detector elements.

In another aspect of this invention, improved processes for forming a matrix hybridization system are described. In one process, a substrate, such as silicon, is formed with an insulating layer, such as a thick oxide. Conductive microlocations are formed, such as by deposition of metal (e.g., aluminum or gold) that is then patterned, such as by conventional photolithographic techniques. An insulating coating is formed, such as TEOS formed by PECVD. Optionally, a nitride passivation coating is formed over the TEOS layer. Openings to the microelectrode are formed through the nitride and glass.

Fig. 6 shows a plan view of the system including an electronic cell sorter matrix, DNA selectors and restriction fragment selectors and hybridization matrix.

Fig. 7 shows a block diagram description of the control system.

Fig. 8 is a cross-sectional view of the active, programmable matrix system having associated electronics.

Fig. 9 is a cross-sectional view of an alternative, layered active, programmable matrix system having electrical and optical access to the backside of the microlocations and a biological containment cover.

Fig. 10 shows a perspective view of the APEX system mounted in a mating carrier.

Figs. 11A-G show process steps in device fabrication.

Fig. 12 shows a fabricated device utilizing a polysilicon structure in cross-section.

Fig. 13 shows a fabricated device utilizing adhesion enhancing layers in cross-section.

Fig. 14 shows a device having an enlarged reservoir space above the electrode.

Fig. 15 shows user displays for various voltage and current regimes.

Fig. 16 shows a cross-sectional view of a DNA purification system.

Fig. 17 shows a cross-sectional view of a capillary array manufacturing system and apparatus.

Fig. 18 shows a perspective view of a micro-location of a concentric structure.

Detailed Description of the Invention

Figs. 2A and 2B illustrate a simplified version of the active programmable electronic matrix hybridization system for use with this invention. Generally, a substrate 10 supports a matrix or array of electronically addressable microlocations 12. For ease of explanation,

contacts the attachment layer 16A as a result of its movement under the electrophoretic force, the functionalized specific binding entity 20 becomes covalently attached to the attachment layer 16A.

It is possible to protect the attachment layers which are not subject to reaction, such as 16B and 16C by making their corresponding electrodes 12B and 12C negative. This results in electrophoretic lines of force emanating from the attachment region 16B (only 16B will be discussed for simplicity, the results being similar for 16C). The electrophoretic force lines 24 serve to drive away negatively charged binding entities 20 from the attachment layer 16B and towards the attachment layer 16A. In this way, a "force field" protection is formed around the attachment layers 16 which it is desired to have nonreactive with the charged molecules 20 at that time.

One highly advantageous result of this system is that charged binding materials 20 may be highly concentrated in regions adjacent to signal attachment layers 16. As can be seen in perspective drawing Fig. 2B, if a individual microlocation 26A is positively charged, and the remaining microlocation are negatively charged, the lines of electrophoretic force will cause transport of the net negatively charged binding entities 20 toward the microlocation 26A. The microlocation 26A is intended to depict the combination in Fig. 2A of the attachment layer 16, the permeation layer 14 and the underlying associated electrode 12. In this way, a method for concentrating and reacting analytes or reactants at any specific microlocation on the device may be achieved. After the attachment of the specific binding entities 20 to the attachment layer 16, the underlying microelectrode 12 may continue to function in a direct current (DC) mode. This unique feature allows relatively dilute charged analytes or reactant molecules free in solution to be rapidly transported, concentrated, and reacted in

way, the concentration aspect is utilized to provide high concentrations at that specific attachment layer then subject to the positive electrophoretic force. The concentrated materials may next be moved to an adjacent, or other, attachment layer 16. Alternatively, multiple attachment layers 16 may be deprotected in the sense that there is a net electrophoretic force field emanating from the electrode 12 through the attachment layer 16 out into the reservoir 18. By deprotecting multiple attachment layer 16, multiplex reactions are performed. Each individual site 26 may serve in essence as a separate biological "test tube" in that the particular environment addressed by a given attachment layer 16 may differ from those environments surrounding the other attachment layers 16.

Fig. 3 shows a plan view of the metal mask layer for an active programmable electronic matrix system. A plurality of individual electrodes 30 are formed preferably in an array. For example, an 8 x 8 matrix of individual electrodes 30 is formed. Optionally, additional control or dump pads 32 may be provided to aid in generation of desired electrophoretic fields. The electrodes 30 and pad 32 are connected to contact pads 34. 68 contact pads 34 are shown corresponding to the 64 electrodes 30 and 4 pads 32. Leads 36 connect the electrodes 30 and pads 32 individually to the contacts 34. As shown, a fan-out pattern is used to permit connections from the relatively condensed region of the electrodes 30 and pads 32 to the boundaries 36 of the mask.

Fig. 4 shows an exploded detail plan view of the mask of Fig. 3. The resulting metallized system would appear substantially similar to the masked pattern. The electrodes 30 are shown formed as substantially square structures. The lead lines 36 connect the electrode 30 to the contact pad 34 (Fig. 3). The preferred line width of the lead 36 is 1 to 20 microns.

groups oe sets of locations can be made selective for one type of cell. Cell selectivity can be imparted by attaching specific antibodies or cell adhesion factors to the attachment layer. The matrix 66 operates by free field electrophoresis.

The crude DNA selector 68 and restriction fragment selector 70 serve to bind the crude DNA output from the electronic cell sorter matrix 66 and permit selective cleavage of the desired DNA from the bound material. The term crude is used merely to denote a non-final stage in DNA isolation or complexity reduction. The DNA is bound to the selector in a region which is believed not to contain the desired DNA material. The desired DNA materials are then severed from the bound materials, such as by application of restriction enzymes. The severed, unbound material is then physically moved from the crude DNA selector 68 to the restriction fragment selector 70. Preferably, electrophoretic transport is used to remove the severed material. This process may be repeated by binding the severed material to a selector, upon which a restriction enzyme acts so as to cleave the unbound portion which contains the desired DNA.

For example, human DNA contains approximately 100,000 genes. Of the total DNA material, a significant portion constitutes repeating sequences which do not contain the desired DNA information. The DNA may be bound to a selector by these noninformation bearing repeating sequences. The bound DNA may be severed from the unbound DNA which is believed to contain the desired DNA to be analyzed. This process may then be repeated with yet more specific sequences causing binding of the material to the selector.

The output of the restriction fragment selector 70 is then supplied to the APEX chip 72. Operations on the matrix 72 are performed as described in connection with Figs. 2A and 2B.

nating or pulsed current at the strip electrode. This drives DNA on and off of the stationary phase.

A further alternative method for reducing the complexity of a sample of DNA, is to size select by sieving the sample through a microporous media. Microporous media can be formed by filling cavities of arbitrary geometry with dendrites. These dendrites are formed by electrochemical deposition of chemicals such as, but not exclusive to, metal salts, ceramic forming materials, monomers and polymers. The porosity of the microporous media can be controlled by adjusting the electrical signal that is applied to the electrodes. For example, dendrites can form picket fence type structures or fractal type structures.

A method for forming microporous media on an APEX device could involve forming a long channel with opposing metal electrodes. When this channel is filled with the appropriate chemical and an appropriate electrical signal is applied to the electrodes, dendrites will form in the interstitial space between the electrodes forming a microporous media.

Returning to Fig. 6, an electronic reagent dispenser system 74 may be provided to deliver reagents to the system 60. Preferably, the reagents are delivered by electrophoretic force if they are charged. Optionally, an electronic waste disposal system 76 is included within the system 60. The waste disposal system 76 attracts charged waste particles to it and disposes of them by holding the charged entities on it. Another optional member of system 60 is the DNA fragment storage system 78. This fragment storage system 78 serves to temporarilly hold DNA fragments for future analysis..

The system 60 may include some or all of the functions described above. For example, the combination of sample preparation in the form of complexity reduction, as performed by the DNA selector 68 and restriction fragment selector 70 may be associated with the analyti-

Additionally, another relay permits selecting the polarity of the voltages supplied to the APEX system 92 electrodes. Optionally, if multiple source levels are available, such as from a multiple output power supply 86, the specific level to be connected to an APEX system 92 electrode may be set independently of those for the other electrodes.

Thus, as described in connection with Fig. 2A, by placing certain electrodes (e.g., 12B and 12C) at a negative, but lesser potential than electrode 12D, the attachment region 16B and 16C would be protected by the local force field.

The interface 88 may serve to select the desired voltage for the individual electrodes in the APEX system 92. Alternatively, such a different voltage arrangement may be achieved through use of a voltage divider.

In the preferred embodiment, the controller computer 80 is a Macintosh Quadra 950. National Instruments Corporation LabVIEW software is used to provide a software interface for a user to program the devices connected to the APEX and to collect and process data from an assay. National Instruments NuBus boards are used to provide the hardware interface from the Quadra 950 computer 80 to the power supply devices 86 that source potentials and currents and that measure the actual currents and potentials and the results of the assay.

The user controls the assay through a Virtual Instrument created with the LabVIEW software. The virtual instrument provides a user friendly graphical representation of the controls that the user may exercise, and of some of the results of applying these controls to the APEX device to perform an assay. The user interfaces with the Virtual Instrument through the keyboard and mouse (collectively, input 84) of the Quadra 950 computer 80. The Virtual Instrument provides software interfaces to a National Instruments NB-MIO-16XL multipurpose input/output 90 and to a National Instruments DMA2800

ments SCXI Chassis with nine 16-channel, Class 3 Relay Modules connected in the chassis, providing a total of 144 relays. Two relays are used per electrode to provide for electrode disconnected or electrode connected to either positive or negative source. In the preferred embodiment, a bundle of cables connects these relays to the APEX device through a Cerprobe Probe Card that provides mechanical contact of probes to the bond pads of the APEX device.

The controller computer 80 optionally controls the illumination source 94 for excitation of fluorescence to detect DNA hybridization. In the preferred embodiment, the illumination source 94 is a laser which outputs radiation at an appropriate wavelength to excite fluorescent markers included within the APEX system 92.

The output of the APEX system 92 is passed through observation path 96 to the detector 98. The observation path 96 may be a physical connection, such as through a fiber optic, or may comprise an optical path such as through a microscope. Optical filters may be utilized in the observation path to reduce illumination of the detector at wavelengths not corresponding to the emission spectra of the fluorescent markers in the APEX system 92. Additionally, notch filters may be utilized as necessary to reduce illumination of the detector 98 at the excitation wavelength of the laser illumination source 94. The detector 98 may optionally form an image of the APEX system 92, such as through the use of a cooled CCD camera. In addition to, or as an alterative to, forming an optical image, the emitted fluorescence radiation from the APEX system 92 may be detected by conventional means such as photodiodes or photomultiplier tubes. The output of the detector 98 is provided to the data processing/analysis system 100. This system monitors the level of detected probe material in the APEX system 92. Optionally, an expert system may be utilized in the analysis system 100.

provide convenient activation paths for the individual circuit elements 114.

Waveguides can be used for guiding excitation light to micro-locations, and for guiding fluorescence signals to detectors. Waveguides can be free standing, as in an optical fiber, or can be integrated into a monolithic semiconductor device. Waveguides can be fabricated from materials such as zinc oxide or indium tin oxide that are also electrically conductive. The waveguide can then serve as both an electrode and as means for transporting optical radiation. Waveguides can be located in or around the plane of the capture probe to minimize nonspecific background fluorescence. Waveguides can incorporate holographic optical elements. The function of these holographic optical elements includes, but is not exclusive to, notch filters, dichroic mirrors, band pass filters, beam splitters, neutral density filters, half-wave plates, quarter-wave plates, polarizers, and lenses.

Fig. 9 shows in cross-section an alternative, layered structure for the active programmable matrix system. In a first layer, individual electrodes 120 are formed upon a support 122. The support 122 is preferably insulating. Above the electrodes 120 is preferably formed a permeation layer 124 and individual attachment layers 126 corresponding to the individual electrodes 120. Optical paths 128 are provided through the support 122 to access the electrode 120. Preferably, the optical path 128 is comprised of a fiber optic or light guiding pipe or structure. Optionally, electrical connection 130 passes through the support 122 to access the electrodes 120 from the backside. The term backside is used herein to connote that side of the electrode 120 which contacts the support 122. In a second layer a semiconductor support 132 includes circuit elements 133 connected to the conductor 131. The conductor 131 is designed such that its upper surface mates and forms

Active, programmable matrices of micro-locations can also be formed from capillary tubes. Fig. 17 shows a system and product formed therefrom. Capillary tube matrices are formed by stacking capillary tubes in arrays 220 of arbitrary geometry, or by melting by heater 222 and drawing, such as by die 224, these arrays 220 into an adherent and integral unit 226. Alternatively, solid rods composed of two diffenent materials arranged about each other concentrically can be used instead of capillary tubes. Fig. 18 shows such a structure in perspective. Here, the material that composes the inner core 230 is etched out from the outer material 234 selectively to form a hole 232 that goes partially or all the way through the device. Alternatively, the inner core may be etched in such a way as to form a controlled porousity glass.

Individual capillary tubes can be addressed by wires inserted into the capillary tube, or by affixing the capillary tube matrix to a complimentary matrix of lithographically formed electrodes. Additionally, the inner cores of the solid rods may be formed from a conducting material. Electrical contact can be made with the inner core material by affixing the solid rod matrix to a complimentary matrix of electrodes, or by lithographically forming electrodes on the solid rod matrix.

The capillary tubes and etched solid rods are filled with an appropriate material to form a permeation layer. The surface of the permeation layer can be functionalized with specialized attachment chemistry.

An alternative method to electrophoretic transport is to use convective mass transport to transport material to microlocations. One device that can accomplish this is a rotating disk. Here convection is acheived by the hydrodynamic shear forces present at boundary between the spinning disk and the solution. Fluid flows straight onto the surface from the bulk solution. A matrix of electrode pads can be attached to a spinning

layer 156. The sequence of preferred steps is as follows. First, the semiconductor, preferably p-type, test grade silicon is oxidized with a thick (10,000 Å) oxide. A conductive polysilicon layer, preferably a polysilicon doped 5,000 Å thick layer is formed. The polysilicon is then patterned, preferably by photolithography using a wet etch. Next, a glass layer, such as PECVD deposited TEOS is formed. A layer approximately 3,000 Å formed at 475°C is preferred for improved adhesion. The glass layer is then patterned, again, preferably with photolithographic techniques using a wet etch. A metal layer is then formed over the surface, preferably by sputtering aluminum to a thickness of 3,000 Å. The metal is then patterned, again preferably photolithographically, with a wet etch. Next, a nitride layer is formed, preferably via PECVD at 70°C to a thickness of 3,000 Å. Next a via is formed photolithographically using a wet etch so as to contact the electrode.

Fig. 13 shows a structure having improved adhesion of metal conductor to underlying insulating layers through the use of an intermediate adhesive metal such as titanium tungsten. A semiconductor 170, preferably silicon, has disposed thereon an oxide layer 172. An intermediate electrode layer 176, formed of a conductive metal such as gold or aluminum, is sandwiched between titanium tungsten 174 and 178. Adhesive metal layer 178 contacts the external electrode 184, preferably formed of platinum. A glass layer 180, such as formed from TEOS, underlies a external nitride coating 182.

Fig. 14 shows a cross-sectional view of an improved electrode arrangement. An electrode 190 is disposed adjacent to an insulator 192, preferably an oxide. A nitride layer 194 overlies the insulator layer 192. Preferably, the nitride layer 194 is undercut such that the insulating layer 192 is set back from the edge 198 of the nitride layer. A reservoir 196 is thereby de-

was mixed into the acrylamide/copolymer formulation to provide a means for attaching specialized functionality to the surface of the permeation layer. The mixture was cast onto the surface of the micro-location. It was then photopolymerized by ultraviolet light. In some cases, AuCl4 was added as a photoinitiator. The polymer formulations were cast from water and the nonaqueous solvents, methanol, tetrahydrofuran, acetonitrile, acetone, and mixtures of these solvents.

DNA capture probe was attached to the surface of the permeation layer by a Schiff base reaction between an oxidized ribonucleoside attached to the DNA capture probe and the primary amine of the poly-l-lysine. This provides evidence of covalent attachment of special functionality to the surface of the permeation layer.

An oxidized DNA capture probe was brought to a surface micro-location by electrophoretic transport. The capture probe was labeled with a fluorescent marker. This demonstrates the ability to address a micro-location by electrophoretic transport.

An oxidized capture probe with a fluorescent marker attached was attracted to the surface of the permeation layer at a micro-location by electrophoretic transport. The permeation layer was removed from the micro-location by mechanical means. No evidence of the presence of the fluorescently labeled capture probe was observed. This demonstrates the ability of the permeation layer to protect the DNA from the electrode surface.

The maximum DC current density that was attained at a gold micro-location, which was not modified with a permeation layer, before bubbles due to water hydrolysis appeared was 8 milliampheres/cm2. The maximum DC current density that was attained at a gold micro-location, which was modified by an acrylamide-based permeation layer, before bubbles due to water hydrolysis appear was 40 milliampheres/cm2. This demonstrates the ability of the permeation layer to raise the maximum accessible

cases, the boxes show the programmed current or voltage as a dotted line, and the measured current or voltage as a solid line.

In addition to the preferred embodiment of the invention and the alternatives described above, several more alternatives are possible. For example, the electric field that gives rise to ion migration may be modulated in time as long as a DC bias voltage or current is applied simultaneously. The use of an AC signal superimposed on a DC bias voltage or current can achieve three things, 1) minimize the background due to nonspecifically bound DNA, 2) provide a means of electronic stringency control where the control variable is the frequency of the alternating current or voltage, 3) provide a means of aligning DNA molecules spatially.

Many alternatives to the detection of hybridized DNA by fluorescence exist. Most of the alternative techniques also involve modification of capture or target or reporter DNA probes with reporter groups that produce a detectable signal. A few of these techniques based on purely physical measurements do not require reporter groups. These alternative techniques are catalogued as follows: (1) Linear Optical Methods including fluorescence, time modulated fluorescence, fluorescence quenching modulation, polarization selective fluorescence, absorption, specular reflectance, changes in index of refraction, ellipsometry, surface plasmon resonance detection, chemiluminescence, speckle interferometry and magneto-optic Kerr effect; (2) Nonlinear Optical Methods including second harmonic generation, third harmonic generation, parametric mixing, optical heterodyne detection, phase conjugation, soliton damping and optical Kerr effect; (3) Methods Based on Thermal Effects including differential scanning calorimetry, multifrequency differential scanning calorimetry, and differential thermal analysis; (4) Methods Based on Mass Changes including crystal microbalances, cantilever

Claims:

1. An electronic device adapted for performing molecular biological processes comprising:

a support having a first generally planar surface,

a plurality of self-addressable electrodes disposed on the first surface of the support, the electrodes having a contacting portion adjacent the first surface of the support, and

individual electrical connections to the electrodes.

2. The electronic device of Claim 1 wherein the individual electrical connections to the electrodes comprise leads disposed on the first surface of the support.

3. The electronic device of Claim 1 wherein the individual electrical connections to the electrodes are electrical pathways extending from the contacting portion of the electrodes into the support.

4. The electronic device of Claim 1 wherein the support includes an insulator.

5. The electronic device of Claim 4 wherein the insulator is an oxide.

6. The electronic device of Claim 1 wherein the support includes a semiconductive material.

7. The electronic device of Claim 6 wherein the support including the semiconductor further includes an oxide layer disposed thereon.

8. The electronic device of Claim 6 wherein control electronics are included within the semiconductive

a controller adapted to receive user input and provide output including control signals to generate independent electronic environments at the electrodes,

a input system for receiving user instructions connected to the input of the controller,

a generator to provide the desired electronic environment at the electrode, the generator operating under control of the controller output, and

an interface adapted to connect the control system to the active, programmable electronic system.

19. The control system of Claim 18 wherein the generator comprises a power supply.

20. The control system of Claim 19 wherein the power supply is a regulated power supply.

21. The control system of Claim 18 wherein the generator comprises a waveform generator.

22. The control system of Claim 18 wherein the interface comprises a relay system.

23. The control system of Claim 22 wherein the interface system includes relays to provide selective connection from the generator to the electrodes.

24. The control system of Claim 22 wherein the interface includes relays adapted to change the polarity of the output of the generator.

25. The control system of Claim 22 wherein the interface includes relays to provide selective connection to either a first level or second level of a signal.

35. The method of complexity reduction of in a solution containing DNA Claim 32 wherein the severed material is removed through electrophoretic force.

36. A system for performing molecular biology reactions comprising:

an input for receiving a sample containing material to be analyzed,

a sample preparation unit, and

an active, programmable electronic device including a plurality of separately addressable electrodes.

37. The system of Claim 36 wherein the active, programmable matrix comprises an array of electrodes.

38. The system of Claim 36 wherein the active, programmable electrodes further include an attachment layer disposed above the electrodes.

39. The system of Claim 38 wherein the attachment layer includes capture sequences.

40. The system of Claim 36 further including a detector operatively positioned to monitor the active, programmable device.

41. The system of Claim 40 wherein the detector comprises an imaging system.

42. The system of Claim 41 wherein the imaging system comprises a CCD camera.

43. The system of Claim 40 further comprising an analysis system adapted to receive the output of the detection system.

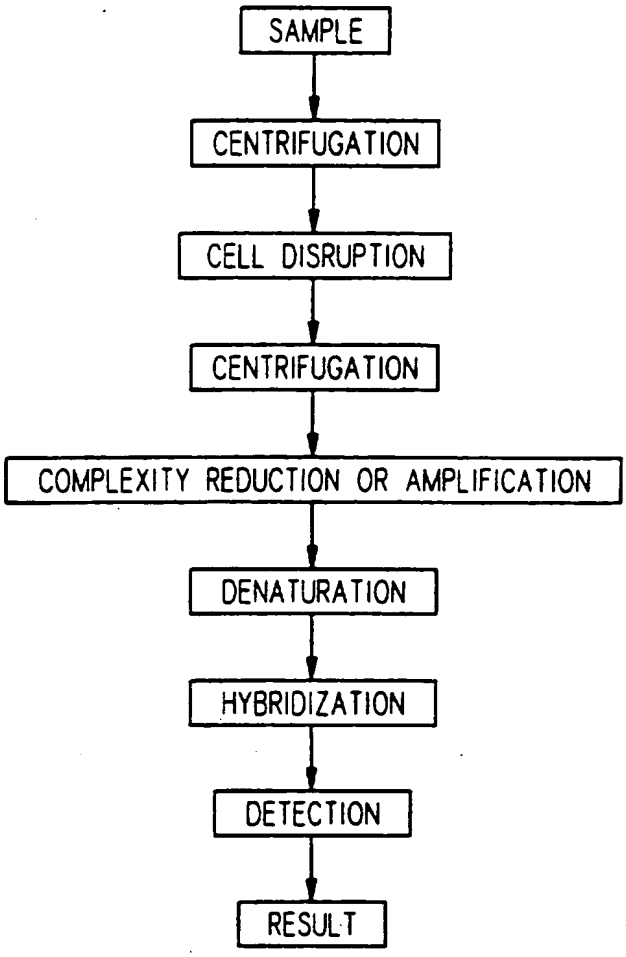

FIG. 1

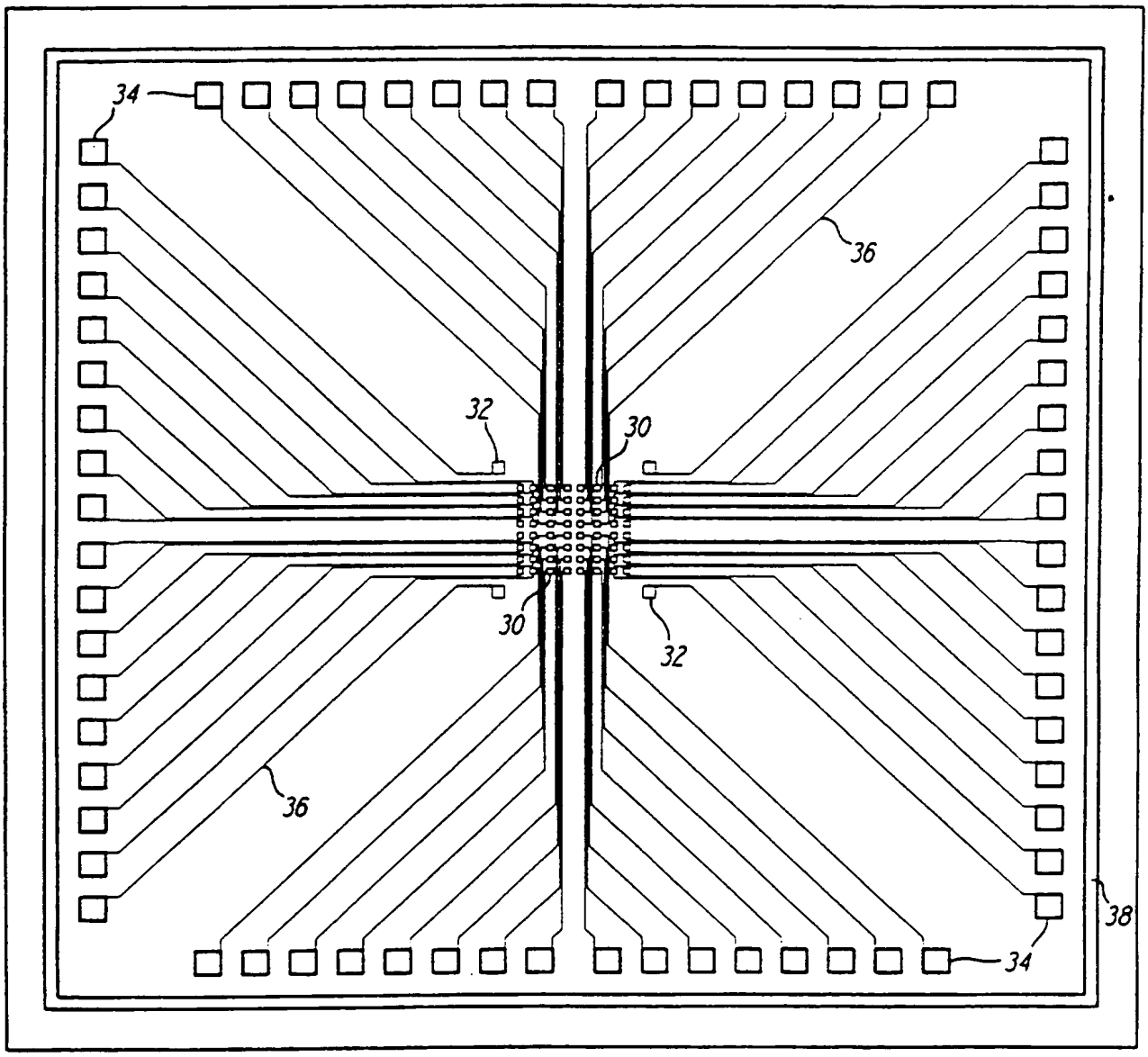

FIG. 3

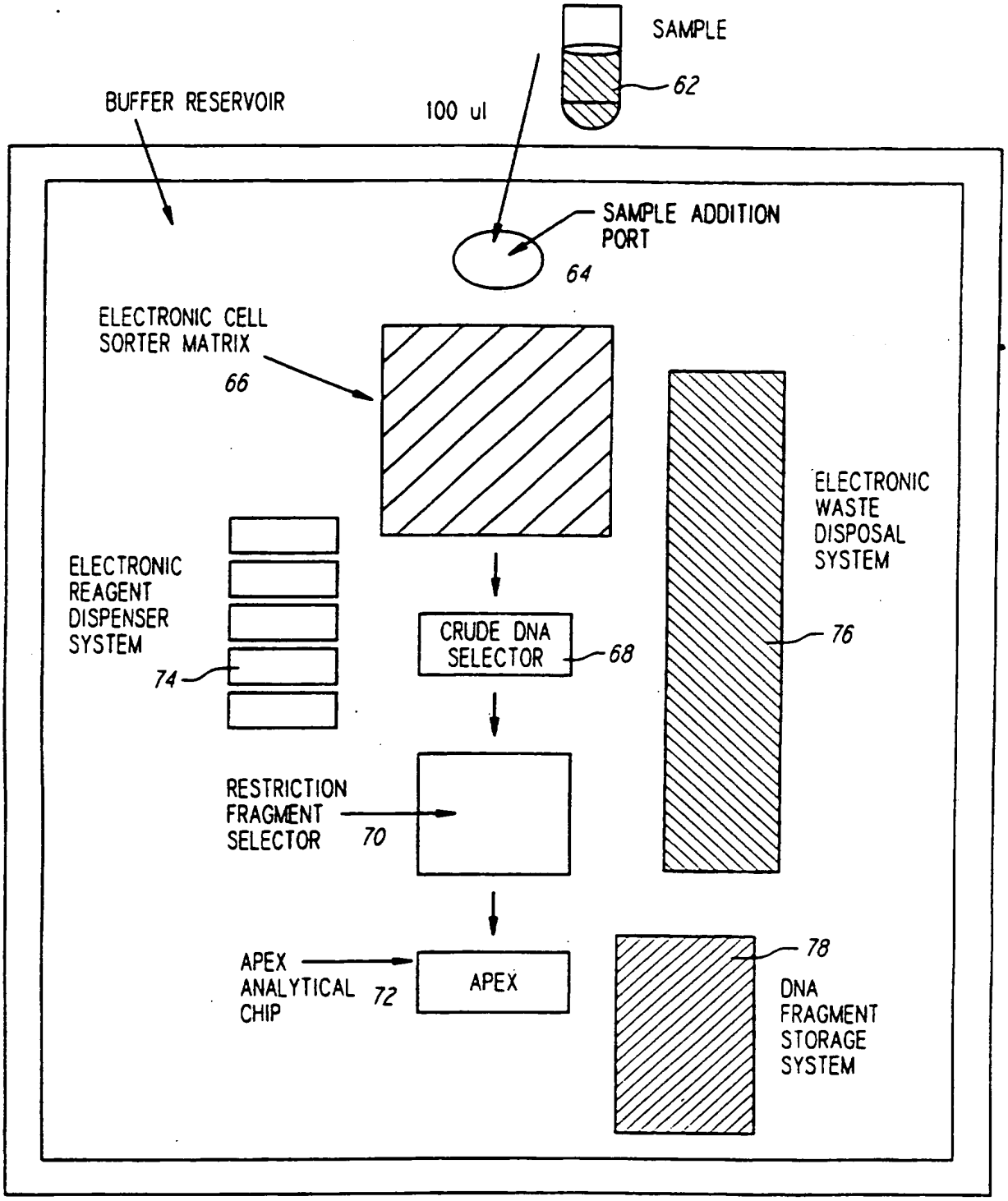

FIG. 6

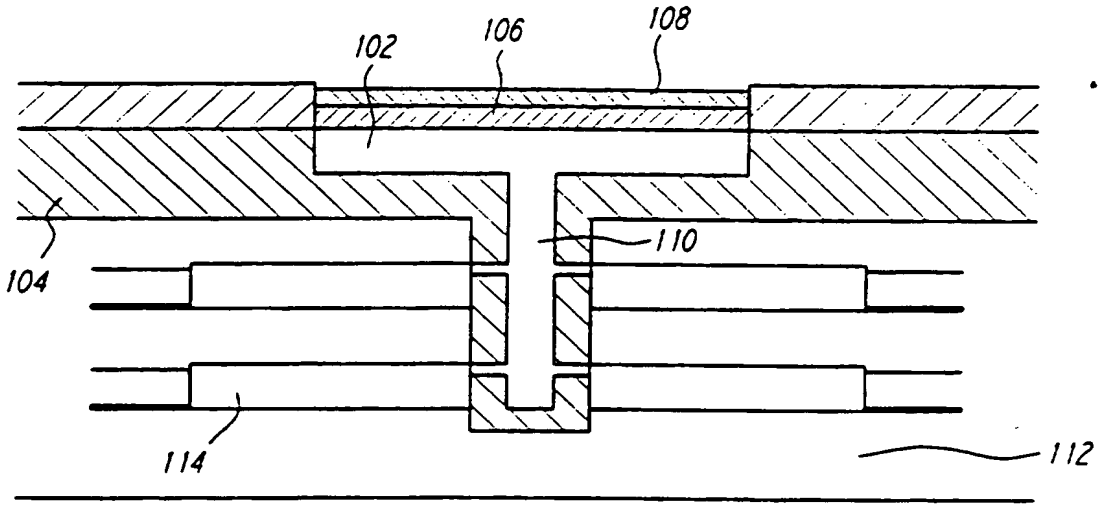

FIG. 8

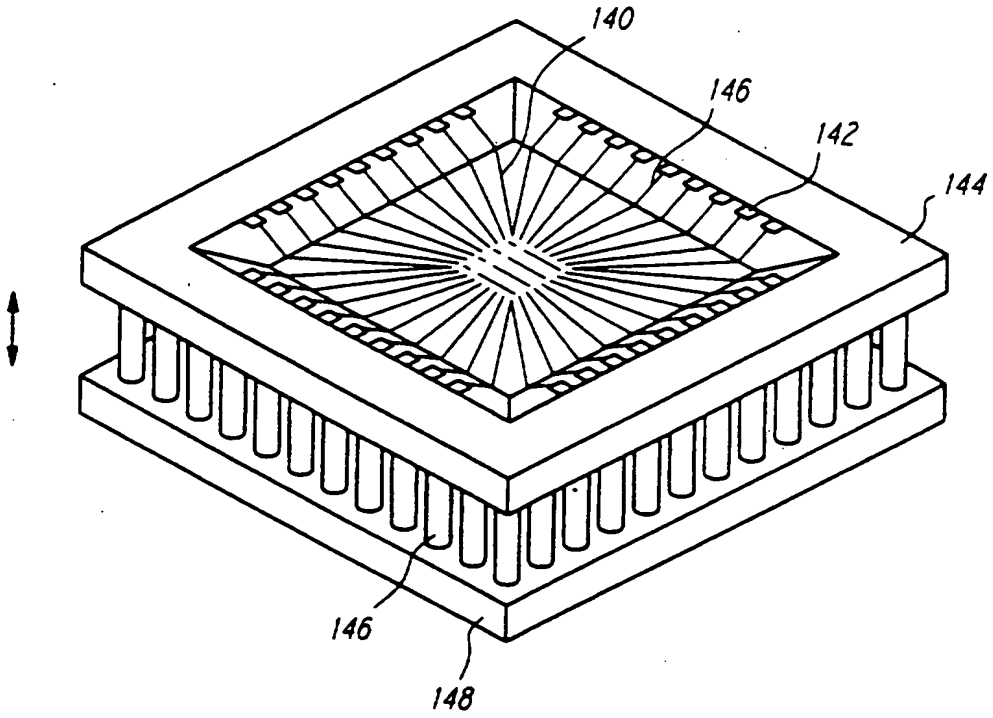

FIG. 10

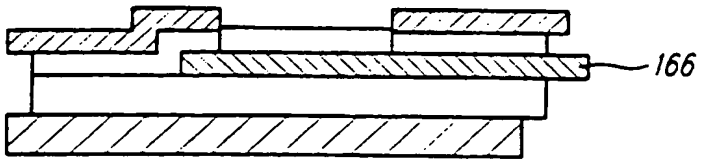

FIG. 12

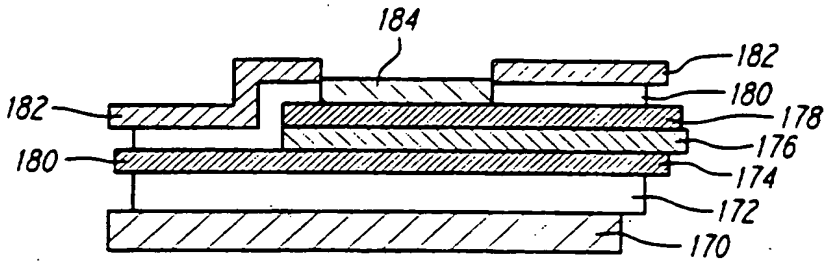

FIG. 13

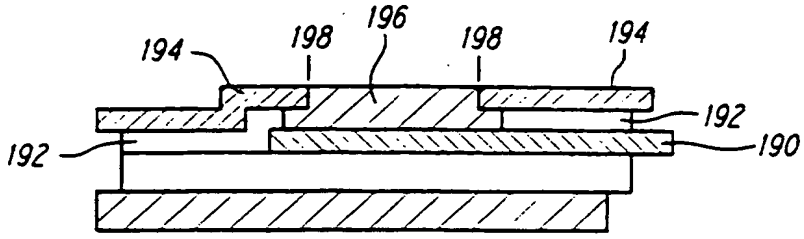

FIG. 14

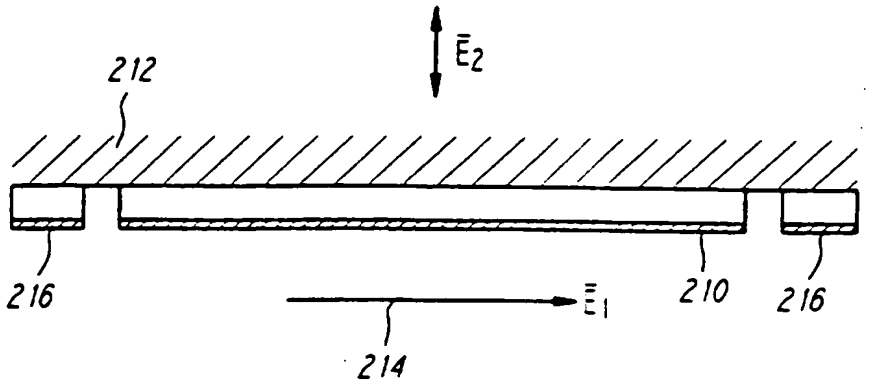

FIG. 16

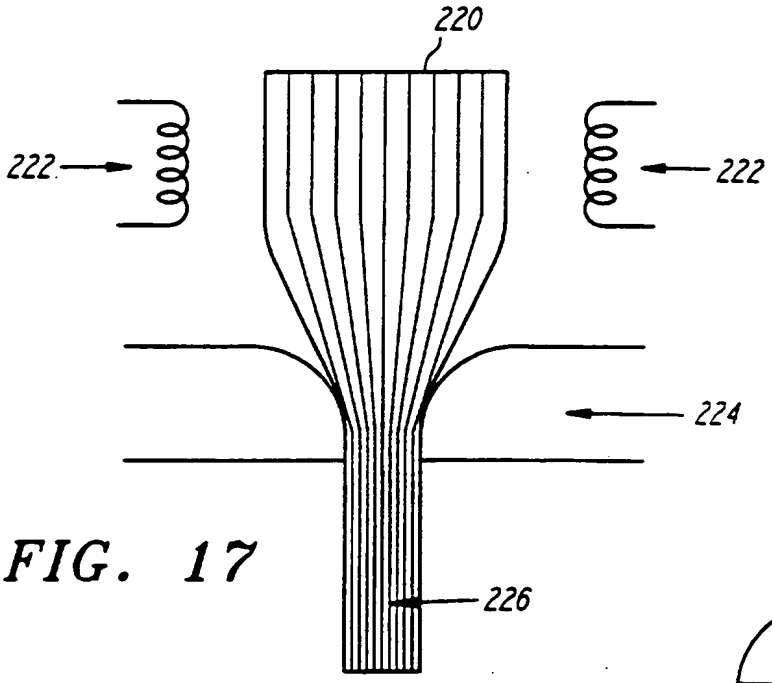

FIG. 17

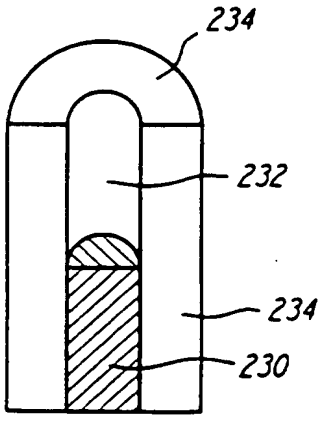

FIG. 18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/11333

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US, A, 5,200,051 (COZZETTE ET AL.) 06 APRIL 1993, see especially the abstract; Figures 4 and 8B and related discussion; column 18, lines 1-2; and column 26, lines 8-10.	1-10,13-31,36-40,43, 49-54 ——— 11,12,41, 42,44-48
Y	US, A, 5,096,669 (LAUKS ET AL.) 17 MARCH 1992, see the entire disclosure and especially claims 1-33.	9-13,17-31,36-54
X — Y	US, A, 5,096,807 (LEABACK) 17 MARCH 1992, see especially the abstract, the Figures, and the discussion related to the Figures.	36,40,41, 43 ——— 1-31,37,42,44-54
X — Y	US, A, 5,227,265 (DEBOER ET AL.) 13 JULY 1993, see especially the abstract, the Figures, and the discussion related to the Figures.	36,40,41, 43 ——— 1-31,37,42,44-54
X, P —— Y, P	US, A, 5,304,487 (WILDING ET AL) 19 APRIL 1994, see especially the abstract, Figures, and claims 1-26.	32,36,40, 41,43-49,51,52,54 ——— 1-31,33-35,37-39,42, 50,53

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